Population connectivity of the corallivorous gastropod *Coralliophila abbreviata*: larval dispersal potential and genetic structure

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Abstract. *Coralliophila abbreviata* (Lamarck) is a coral-eating gastropod found throughout the Caribbean and Western Atlantic. These snails cause substantial and chronic mortality of the two ESA listed 'Threatened' scleractinian coral species, *Acropora palmata* and *A. cervicornis*. The objectives of this project were to 1) determine the species status of *Coralliophila abbreviata* 2) estimate the dispersal potential of *C. abbreviata* veligers by determining the adult reproductive season and frequency, planktonic larval duration, larval settlement behavior and 3) estimate actual gene flow among *C. abbreviata* populations on a Caribbean-wide scale, using novel, highly polymorphic molecular markers (microsatellites).

Eight polymorphic microsatellite loci of *C. abbreviata* were isolated and then characterized using 60 *C. abbreviata* from two geographically disparate populations (Key Largo, FL USA and the Grenadines). All loci were highly polymorphic with an average number of alleles per locus of 24 (range 13-34). Observed and expected heterozygosity values ranged from 0.375 - 0.969 and 0.877 - 0.981, respectively. Three loci deviated significantly from Hardy-Weinberg equilibrium in both populations, presumably due to null alleles. No linkage disequilibrium between pairs of loci was detected

A total of 211 *C. abbreviata* from three coral host taxa and five geographical locations, spanning the species range, have been genotyped using the five loci that conformed to HW expectations. The mitochondrial Cytochrome b gene was sequenced for 82 individuals from two coral host taxa and three geographical locations. No significant genetic structure among hosts or geographically disparate populations was found, indicating that *C. abbreviata* is one plastic species with high population connectivity.

Settlement assays were unsuccessful as culturing and raising *C. abbreviata* veliger larvae to competence and metamorphosis proved difficult. However, we did maintain a batch of larvae swimming in the water column for approximately 32 days. Little shell growth and no metamorphosis were observed. Such a protracted pelagic larval duration may be suitable to explain the lack of genetic structure observed for this species. Monthly *in situ* sampling of female *C. abbreviata* to ascertain reproductive patterns was abandoned as we wished to collect only one or two females from each coral colony throughout the study period to avoid complications with socially induced sex

change of these sequential hermaphrodites and we could not locate enough infested *Acropora palmata* colonies in the study area support this sampling scheme. Therefore, we were only able to collect females every one to two months for a six month period. Females reproduced throughout the observed six month period and, although the degree of difference was variable, the proportion of females brooding egg capsules was consistently lower for snail populations found on *Montastraea* spp. than on *A. palmata*. These data support the hypothesis that snail populations feeding on acroporid corals have higher fitness than those feeding on other corals.

This project has generated valuable information on the population genetic structure, reproductive patterns and early life-history of *C. abbreviata,* which to our knowledge, has not been previously reported. Furthermore, the first microsatellite markers were developed for *C. abbreviata* and thus constitute a valuable tool set that can be used to address a multitude of ecological and evolutionary questions for this species.

NARRATIVE

Reproductive Season and Frequency

Population dynamics, connectivity, and genetic structure of a species are all highly dependent on adult reproductive biology (mode, season, and frequency of reproduction, and fecundity). For *C. abbreviata*, brood size increases as a power function with female shell length (Johnston and Miller 2007). Females feeding on acroporid corals reach larger sizes than those feeding on other prey species (Hayes 1990; Bruckner et al. 1997; Baums et al. 2003; Johnston and Miller 2007) and thus, on average, produce more offspring (Johnston and Miller 2007). However, very little is known about the reproductive patterns of *C. abbreviata* such as seasonality or frequency of reproduction or the variation in these patterns among snail populations on different coral hosts. The objective of this component of the project was to assess seasonal and host specific patterns in reproduction of *C. abbreviata*.

The goal was to collect female snails monthly for 12 months from two coral host taxa (*A. palmata* and *Montastraea* spp.) from multiple sites in the Florida Keys National Marine Sanctuary (FKNMS) to determine their reproductive status. However, to avoid complications with socially induced sex change of these sequential hermaphrodites, we did not want to resample groups on individual coral colonies during the study period. We thus had to depart from the planned monthly sampling scheme as we could not locate enough *Acropora palmata* colonies in the study area that harbored snails that were not part of a long term monitoring project that requires that snail populations remain in tact. Female snails were thus only collected in June, August, September, and November of 2007, from 10 sites in the FKNMS (Table 1). At each site, SCUBA divers searched *Acropora palmata* and *Montastraea* spp. colonies for snails. *C. abbreviata* are protandrous hermaphrodites that exhibit host and sex specific variation in size frequency distributions: snails on *A. palmata* are larger than snails on *Montastraea* spp., and females are larger than males on both host taxa. Thus, to increase the likelihood of collecting females, only snails larger than 30 and 20 mm shell length were collected from

A. palmata and *Montastraea* spp., respectively (size ranges are based on data from Johnston and Miller 2007). Only 1-2 snails were collected from any one coral colony. Because female *C. abbreviata* often expel egg capsules during collection, snails were taken off the coral and placed immediately into individual containers for transport back to the laboratory. At the laboratory, shell length was measured with vernier calipers to the nearest 0.01 mm. Shells were crushed and the soft tissue was examined to confirm sex (absence of penis above the right eye stock or presence of egg capsules in the mantle cavity). The presence and number of egg capsules was recorded along with the developmental stage of the embryos. The following criteria were used to classify embryonic development into four sequential stages: 1.) yellow egg mass concentrated at one pole of the transparent egg capsule (early); 2.) larger yellow embryos diffuse throughout the egg capsule (mid-early); 3.) orange embryos (mid-late); 4.) dark orange/brown swimming embryos filling the egg capsule (late). Stages 1 and 2 and 3 and 4 were then grouped into early and late stage embryos, respectively (Fig. 1). The proportion of females brooding egg capsules was averaged over sites for each coral host taxa and sampling time and compared between hosts and among months using two-way analysis of variance (ANOVA; Fig. 1). Female snails from both coral hosts brooded egg capsules at every sampling time. There was no significant effect of sampling date (p = 0.562), but there was a significant effect of coral host ($p < 0.01$) and a significant host*date interaction ($p = 0.037$) on the proportion of females brooding egg capsules (Table 2; Fig. 2).

Although higher frequency sampling combined with non-destructive monitoring of individual snails is needed to fully elucidate the reproductive season, number of broods per year, and the length of intracapsular development for *C. abbreviata*, these data provide some first insight on host-specific reproductive patterns of *C. abbreviata*. Females reproduced throughout the observed six month period and, although the degree of difference was variable, the proportion of females brooding egg capsules was consistently lower for snail populations found on *Montastraea* spp. than on *A. palmata*. These data support the hypothesis that snail populations feeding on acroporid corals have higher fitness than those feeding on other corals.

Date	Site	*A.palmata*		*Montastraea* spp.	
		N_f	Brooding (%)	N_f	Brooding (%)
6/20/2007	EL	7	100	4	75
	DR	4	100	5	60
	LG	5	100	6	66.67
	ML	3	100	5	60
8/23/2007	EL	6	100	5	60
	GR	6	66.67	4	100
	CF	4	50	4	75
	TR	6	83.33	4	50
9/22/2007	GR	3	100	5	60
	SI	6	100	4	50
	TR	0	N/A	4	50
	WA	4	100	4	25
11/30/2007	DR	1	100	4	75
	EL	4	100	4	25
	FR	3	66.67	5	40
	LG	0	N/A	4	75

Table 1 Number of female *C. abbreviata* (N_f) collected from two coral host taxa (*A. palmata* and *Montastraea* spp.) at each site and sampling time and percent of those females brooding egg capsules. Reef sites in the FKNMS include Elbow (EL), Key Largo Dry Rocks (DR), Little Grecian (LG), Molasses (ML), Grecian Rocks (GR), Carysfort (CF), Turtle Rocks (TR), Sand Island (SI), and French (FR).

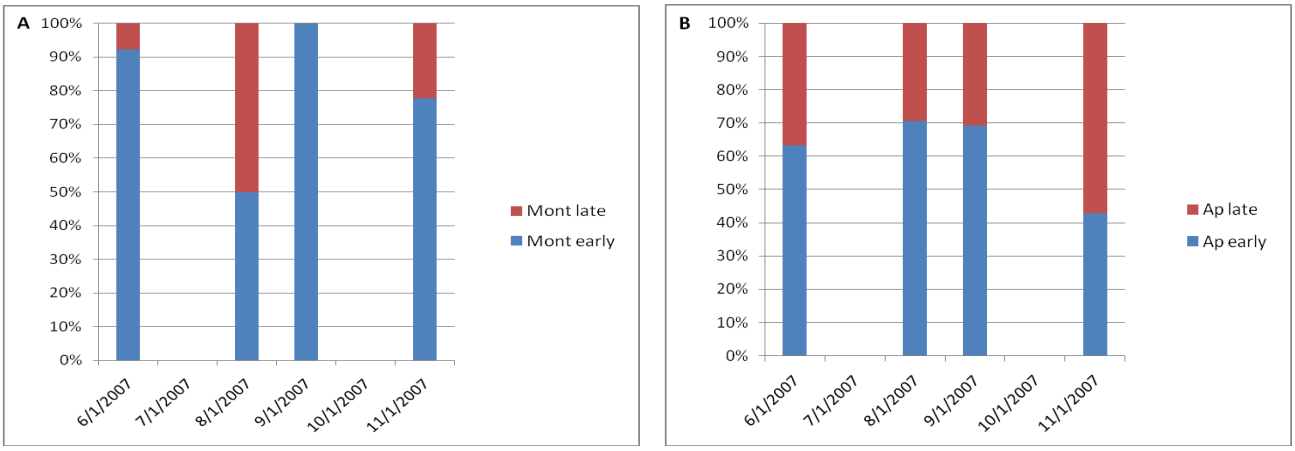

Fig. 1 Percent of embryos in early (blue) and late (red) stages of development. Embryos from female *C. abbreviata* found on *Montastraea* spp. (A) and *A. palmata* (B) corals.

	SS	DF	MS	F	p
Date	558.2	3	186.1	0.6979	0.562488
Host	8515.1	1	8515.1	31.9381	0.000008
Date*Host	2650.4	3	883.5	3.3137	0.037016
Error	6398.7	24	266.6		

Table 2 Two-way ANOVA. The effects of coral host and date on the proportion of female *C. abbreviata* brooding egg capsules.

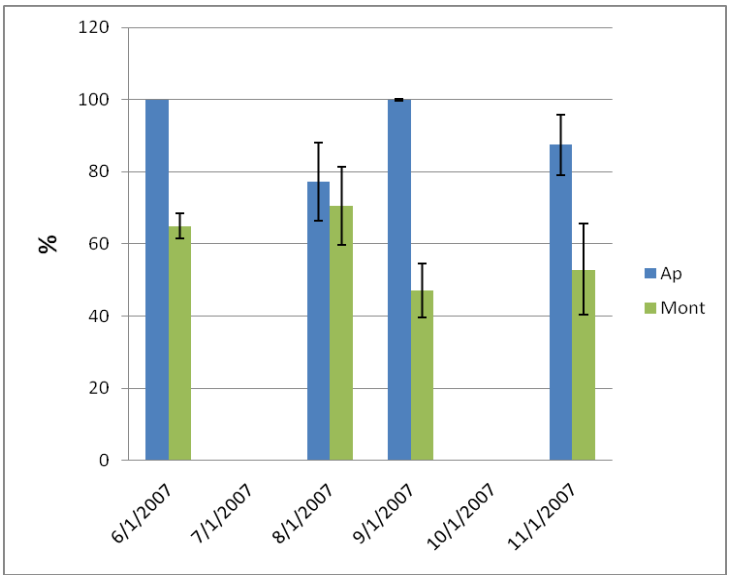

Fig. 2 Percent of female *C. abbreviata* from each coral host taxa, brooding egg capsules (averaged across sites ± 1 StdErr). See Table 2 for ANOVA results.

Larval Growth and Pelagic Duration

Connectivity of populations of benthic marine organisms is often dependent on the dispersal of planktonic larvae. Planktonic larval duration (PLD) is a key determinant of dispersal distances and patterns for these organisms and is, therefore, necessary to parameterize biophysical dispersal models (see Cowen et al. 2006; Levin 2006). Understanding dispersal patterns is crucial to understanding the dynamics and stability of natural populations. The goal of this project was to assess the growth rate and determine the PLD of *C. abbreviata* veliger larvae.

Many attempts were made under various culturing conditions to raise *C. abbreviata* veliger larvae to competence and metamorphosis. Larvae were maintained at various densities, in still, rolled, bubbled, and re-circulating sea water systems with and without algae and antibiotics and with various levels of filtration. Most larval cultures crashed (90-100% mortality) within two weeks. However, one batch of larvae was maintained for approximately 32 days. These larvae were maintained in 1L roller bottles at 23°C with 32ppt filtered seawater (fsw) and a mixture of antibiotics and unicellular algae. Water was changed every 2-3 days. The maximum shell length of a subset of 20-25 veligers was measured periodically over a four week period (Fig. 3). The velar lobes grew noticeably over this period, but there was little shell growth and no metamorphosis was observed. The heart, stomach, and intestines, were all visible through the transparent shell of the veligers. Dark material moving through the stomach and intestines indicated that the veligers were indeed feeding on the algae provided. Poor growth in this culture could potentially be an effect of temperature. The larvae were maintained indoors at 23°C, whereas the average sea surface temperature on the reef tract off of Key Largo at that time of year is approximately 28°C. Unfortunately, incubating or heating the roller bottles was not possible. To test for potential competency of these larvae, at five weeks post-hatch, approximately 10 larvae were placed in each of two Petri dishes containing

either an *Acropora cervicornis* or *Montastraea faveolata* coral fragment. No metamorphosis was observed in these treatments after 48 hours.

Although we were unable to determine the exact PLD and size at settlement, 32 days is the longest reported period that *C. abbreviata* veliger larvae have been kept alive and swimming in culture. Such a protracted larval duration, in combination with a high frequency of reproduction (above) might be suitable to explain the lack of genetic structure that we observed over a large geographical range (see below). These results provide initial observations of the early life history of *C. abbreviata* and baseline for future work.

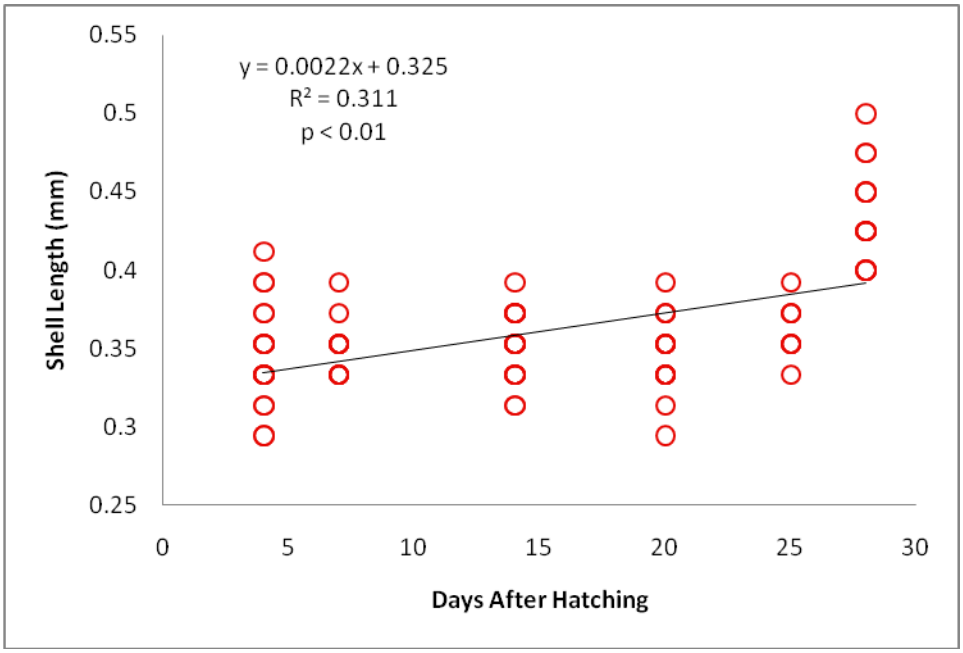

Fig. 3 Growth of *Coralliophila abbreviata* larvae. Veliger larvae were obtained from late stage egg capsules that were expelled from multiple female *C. abbreviata* on June 7th, 2007 (hatch date). The maximum shell length of 20-25 larvae was measured at each sampling day.

Settlement Behavior and Cues

Actual dispersal and recruitment of benthic marine organisms requires that larvae come in contact with suitable settlement habitat. Since adult habitat usually represents a relatively small portion of the area that may be encountered while in the plankton, larvae often settle in response to chemical or physical cues that are specific to critical resources such as habitat, food, and adult conspecifics. The specific cue(s) that induce larvae to settle may determine dispersal patterns and population distribution on both a local and regional scale. The goal of this project was determine the settlement cues for *C. abbreviata.*

Ideally, the PLD and time to competence would have been determined in the above component of the project, allowing us to investigate the strength of various potential settlement cues on competent larvae. This was not the case, however, so we decided to test for competence and settlement at short intervals post-hatch. Settlement

assays were conducted with a batch of larvae maintained at 25°C in 5 L of fsw with a mixture of unicellular algae. Water was changed every two days. Assays were conducted in Petri dishes containing 50 ml of fsw. Four potential settlement cues (the corals *A. cervicornis* and *M. faveolata*, the macroalgae *Halimeda* spp., and crustose coralline algae) were tested against a control (fsw). There were four replicates per treatment and control for a total of 20 dishes per assay. Every four days post-hatch, five larvae were added to each dish and metamorphosis was scored after 48 hours. No metamorphosis was observed after 12 days when the larval culture subsequently crashed.

Culturing techniques for *C. abbreviata* larvae should be optimized before additional manipulative settlement experiments are attempted. Alternatively, field based assessments of natural recruitment patterns of *C. abbreviata* may shed light on settlement preferences.

Molecular Analysis

Multilocus genotyping approaches can detect gene flow on demographic and ecologically relevant time scales. These methods have proven particularly useful in the study of metapopulation dynamics. Metapopulations are characterized by distinct local populations separated to some extent by unsuitable habitat. The patterns and scale of effective dispersal or connectivity among such patches is often difficult to quantify and has thus been a controversial topic. Coral reefs, for instance, represent a relatively small portion of the marine benthos and can be separated by several meters to thousands of kilometers. Most coral reef organisms, including *C. abbreviata*, have a bipartite life-history, spending days to months in the pelagic realm as larvae before settling to a benthic or demersal existence as juveniles and adults. Historically, it was thought that long-distance dispersal of pelagic larvae via oceanic currents was common and marine populations were, in general, demographically open. Realized dispersal, however, is a complex function of biological and physical processes and may be affected by both pre-settlement planktonic processes such as current regimes and larval behavior as well as post-settlement processes that affect the survival of settlers (see Sponaugle et al. 2002). The small size of many marine fish and invertebrate larvae and the great distances which they potentially travel, make it difficult to quantify dispersal using traditional tracking and sampling techniques. Estimates of actual gene flow from molecular genetic analyses, however, have revealed various patterns of connectivity among coral reefs. In general, although long distance dispersal does occur, local retention of larvae and population subdivision is far more prevalent than previously thought (see reviews in Hellberg 2007; Rocha et al. 2007).

The degree to which populations are opened or closed influences demographic, ecological, and evolutionary processes. High connectivity, which characterizes open populations, precludes local adaptation and may mitigate mechanisms of population regulation on local scales making populations more resilient to local environmental disturbances and population fluctuations (Hixon et al. 2002). Thus, understanding the patterns and mechanisms of connectivity is crucial for the conservation and management of coral reefs, especially the effective design of spatially discrete management units such as Marine Protected Areas (MPAs).

Molecular genetic analyses also play a critical role in clarifying taxonomic uncertainties and identifying cryptic and sibling species. Cryptic and sibling species are genetically distinct, often sympatric species that are morphologically difficult to distinguish and are thus often classified as one species using traditional techniques. Many sympatric sibling species have characteristic differences in behavior, ecology, or life-history, such as specialized food sources or habitats. Cryptic species complexes have already been discovered in most taxa and habitats and it is thought that more research focused in biodiversity hotspots such as tropical rainforests and coral reefs will uncover much more hidden diversity (reviewed in Bickford et al. 2007). The characterization of inter and intraspecific genetic variation is not only important to effectively manage and conserve biological diversity but also to understand interspecific interactions and uncover patterns and mechanisms of differentiation and speciation.

Coralliophila abbreviata populations found on branching acroporid corals display different morphological, behavioral, and life-history characteristics than those found on massive and plating corals (Baums et al. 2003a,b; Johnston and Miller 2007), prompting hypotheses about host-associated genetic differentiation within *C. abbreviata*. Other coral-associated organisms have speciated as a consequence of coral host specialization, in some cases without much morphological differentiation (Gittenberger and Gittenberger 2005; Faucci et al. 2007). *Coralliophila meyendorffii*, a species found in the Eastern Atlantic and Mediterranean, like *C. abbreviata*, has populations characterized by host-specific morphology and life-history. For this species, however, molecular data suggest that host-specific variation is due to phenotypic plasticity rather than genetic differentiation (Oliverio and Mariottini 2001). The species status of *C. abbreviata* has important implications for the management and conservation of imperiled Caribbean corals as it affects population dynamics, patterns of host use, and other community interactions. Under the assumption of one plastic species of *C. abbreviata*, the less effected massive and plating corals could serve as reservoir hosts, producing a continuous supply of predators even as acroporid populations continue to dwindle. Under these circumstances, predation by *C. abbreviata* could eventually lead to local population extinctions and/or inhibit the recovery of the Caribbean acroporid corals, *A. palmata* and *A. cervicornis*.

In this study, to provide enough power to resolve phylogenetic relationships between closely related species as well as intraspecific population genetic structure and gene flow, a combination of microsatellite and mitochondrial molecular markers was used. A 366bp portion of the mitochondrial cytochrome b gene (*Cytb*) was amplified and sequenced using published primers (Merritt et al. 1998). Microsatellite markers were developed and characterized here.

Microsatellite development

Genomic DNA was extracted from the foot tissue of snails using a QIAGEN DNeasy Tissue Kit. DNA samples from two individuals collected from Saint Vincent and the Grenadines in the Eastern Caribbean and Lee Stocking Island in the Bahamas were used to construct a genomic DNA library enriched for microsatellite DNA loci containing $(AACC)_n$, $(AACG)_n$, $(AAGG)_n$, $(AAC)_n$, $(AAG)_n$,$(AAT)_n$, $(ACT)_n$, and $(AC)_n$ repeats, using a hybridization-capture technique modified from Glenn and Schable

(2005). The detailed protocol is available at http://www.srel.edu/microsat/Microsat_DNA_Development.html. Briefly, DNA samples were digested for one hour using the restriction enzymes *Rsa*I and *Bst*UI. DNA fragments were then ligated to a double stranded linker (SuperSNX24 Forward: 5'-GTTTAAGGCCTAGCTAGCAGAATC-3' and SuperSNX24+4P Reverse: 5'-pGATTCTGCTAGCTAGGCCTTAAACAAAA-3') with DNA ligase at16 °C overnight. Two mixtures of the 3' biotinylated di, tri, and tetranucleotide repeat oligonucleotides were used as probes to enrich the DNA samples for microsatellite loci. The linker ligated DNA fragments were hybridized to the biotinylated microsatellite probes in a 2X Hyb Solution (12X SSC, 0.2% SDS) by denaturing the mixture at 95°C for 5 minutes, quickly ramping down to 70°C and then stepping down 0.2°C every 5 seconds for 99 cycles, maintaining at 50°C for 10 minutes, then ramping down 0.5°C every five seconds for 20 cycles, then quickly ramping down to 15°C. Enriched DNA fragments were captured using Dynabeads (Invitrogen), recovered by polymerase chain reaction (PCR) using the SuperSNX24 forward primer (5'-GTTTAAGGCCTAGCTAGCAGAATC-3'), and cloned using a TOPO TA Cloning System (2.1) (Invitrogen). Positive colonies were amplified by PCR using M13 forward and reverse primers. PCR products were examined on a 1% agarose gel along with a 100bp ladder. Products in the 500-1000 bp size-range were cleaned using a Montage PCR Cleanup Kit (Millipore) and sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI 3730 sequencer. Sequences were assembled and edited in SEQUENCHER v4.1 (Gene Codes) and then visually searched for microsatellite repeats. Thirteen primer pairs were designed for microsatellite flanking regions using Primer3 software. Primers were initially tested for amplification using the two DNA samples noted above. Amplification products were visualized on a 2% agarose gel with a 50bp ladder. Of the 13 primer pairs, 11 amplified products of the expected size and were labeled with either VIC, 6-FAM, NED, or PET fluorescent dyes on the forward primer. Thirty additional snails collected from Little Grecian reef in the Florida Keys National Marine Sanctuary (N 25° 07.111; W 80° 18.082) were then genotyped. PCR amplifications were carried out in a 10µL reaction volume containing 50-100 ng genomic DNA, 1 X PCR buffer (containing 1.5 mM $MgCl_2$), 0.2 mM of each dNTP, 0.15 µM 5'-labeled forward primer, 0.15 µM unlabelled reverse primer and 1 U Taq DNA polymerase. The following touchdown thermal cycling program was used: 94 °C for five min, followed by three cycles of 94 °C for 15 s, 60 °C for 15 s, 72 °C for 45 s, 12 cycles of 94 °C for 15 s, 60 °C - 54 °C (ramping down 0.5 °C per cycle), 72 °C for 45 s, 25 cycles of 94 °C for 15 s, 54 °C for 15 s, 72 °C for 45 s, and finally 72 °C for 10 min. The PCR products were genotyped on an ABI 3730 sequencer (Applied Biosystems) and analyzed using GENEMAPPER software (Applied Biosystems).

Of the 11 loci, three were found to be monomorphic and are not described further. An additional 30 individuals from St. Vincent and the Grenadines were then genotyped with the remaining eight loci. Characteristics of the eight polymorphic microsatellite loci are given in Table 3. Calculations of the expected and observed heterozygosities (H_E and H_O, respectively) as well as tests for Hardy-Weinberg equilibrium and linkage disequilibrium were performed using GENEPOP v.4 (Raymond & Rousset 1995). After Bonferroni adjustment of α for multiple comparisons, three loci (Ca602, Ca606 and Ca607) had significant heterozygote deficits ($P < 0.006$, $k = 8$). These loci were checked

for the presence of null alleles, stuttering, and large allele dropout using MICRO-CHECKER (van Oosterhout *et al*. 2004) and null alleles were detected for all three loci ($P < 0.05$). GENEPOP could provide no information regarding linkage disequilibrium for loci Ca606 because each individual had a unique genotype. However, no other pair of loci displayed linkage disequilibrium (adjusted $\alpha = 0.002$, $k = 28$). A large effort was made to optimize multiplex reactions for these loci, but only one multiplex reaction with two loci (Ca600 and Ca 601) was successful. Thus, the remaining loci are amplified separately and the PCR products are combined prior to genotyping on the ABI. Furthermore, we are in the process of testing these eight loci for amplification in the congener *C. caribaea*.

The five loci that did not deviate from HW equilibrium have, to date, been used to genotype 211 *C. abbreviata* from five locations and three coral host taxa, throughout the species range (Fig. 4, Table 4). A 366bp portion of the mitochondrial Cytochrome b gene was amplified from *C. abbreviata* from two coral host taxa and three geographical locations (n=82; Table 4). Pairwise Fst values were calculated with microsatellite data (Table 5) and a 95% parsimony network was created for cyt b haplotypes using TCS (Templeton et al. 1992; Fig.5). We have found no significant genetic structure between hosts or among geographic locations, indicating that *C. abbreviata* is one plastic species with unrestricted gene flow throughout the range sampled. However, further analyses of molecular data needs to be conducted to fully elucidate population genetic structure and gene flow.

Table 3 Characterization of eight microsatellite loci isolated from *Coralliophila abbreviata*. * Indicates significant deviation from Hardy-Weinberg equilibrium (adjusted $\alpha = 0.006$). F, refers to the forward primer; R, reverse primer; N is the sample size; N_a is the number of alleles observed; H_E and H_O are the expected and observed heterozygosities, respectively; *P* is the exact *P*-value for the Hardy-Weinberg test with the alternative hypothesis of heterozygote deficit.

Locus	Primer sequence (5'-3')	Repeat motif	Size range (bp)	Pop	N_a	H_E	H_O	*P*
Ca600	F: AAGGCAGAGGGGAAAACAGT	$(CAT)_{17}$	181-235	FL	13	0.877	0.969	0.988
	R: TTACCTGGGGACAACTGGAG			GR	16	0.900	0.821	0.100
Ca601	F: GAGCAGGGTGAAGAAAGACG	$(AAG)_{23}(AGG)_5$	210-401	FL	34	0.981	0.938	0.036
	R: ACCCCTGCAAATTCTCCTTT			GR	34	0.980	0.893	0.006
Ca602*	F: CGTTTGACATAACTGAGCGTTT	$(GT)_{15}(GTT)_6$	192-256	FL	21	0.955	0.656	0.000
	R: GAGCTTGCCAATAAATTGTGG			GR	21	0.951	0.500	0.000
Ca606*	F: GGGAAAGTAGTGTGGTGGACA	$(CTGT)_{14}$	133-244	FL	25	0.971	0.656	0.000
	R: GCCACTTTTCATTCCTAATCCA			GR	20	0.939	0.607	0.000
Ca607*	F: CAAAAGATGTGGCGTCAAAA	$(GT)_{22}$	196-258	FL	18	0.946	0.375	0.000
	R: GCTTCAGTGCGATACACTCG			GR	19	0.952	0.429	0.000
Ca608	F: CTCCTTTCGTCTGGCTATGTG	$(GT)_{26}$	179-253	FL	20	0.912	0.845	0.273
	R: TAATGGGCAGTGGCAATTTT			GR	20	0.933	0.964	0.887
Ca609	F: TTGGTGTTTGTAGGTTTTTGTTC	$(CT)_{22}$	178-264	FL	34	0.977	0.906	0.078
	R: AAAAAGGGAGGGAAAGCAAA			GR	29	0.972	0.929	0.279
Ca612	F: TGGGACAGATGCACAGGTAA	$(GT)_{33}$	291-382	FL	25	0.962	0.969	0.709
	R: TTCAGCAGCGAAAGGTATCA			GR	27	0.965	0.893	0.032

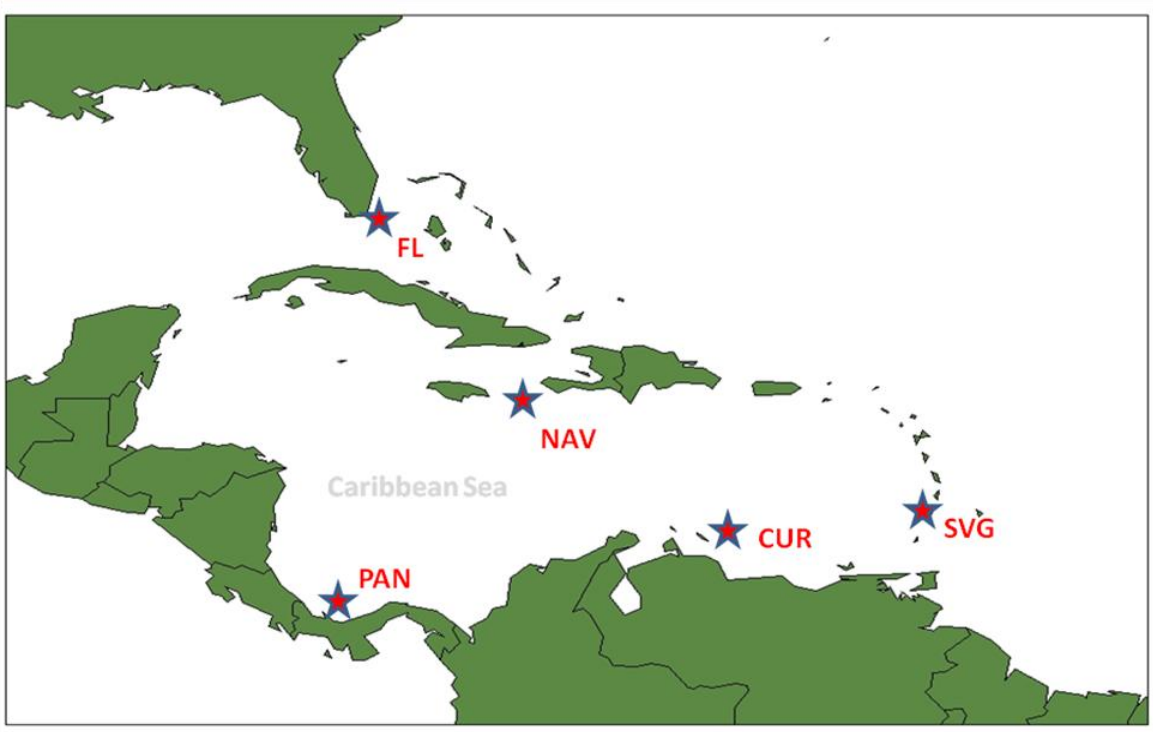

Fig 4 Sampling locations for molecular analyses; Florida (FL), Navassa Island (NAV), St. Vincent and the Grenadines (SVG), Curacao (CUR), and Panama (PAN).

Table 4 Sample size for each location, coral host taxa, and molecular marker used.

Location	Coral Taxa	Code	mtDNA	Msats (n=5)
Curacao	*Acropora*	CA	-	23
Curacao	*Montastraea*	CM	-	34
Florida	*Acropora*	FA	12	22
Florida	*Montastraea*	FM	15	24
Navassa Is.	*A. palmata*	NA	16	14
Navassa Is.	*Montastraea*	NM	12	21
Panama	*Mycetophilia*	PMY	-	28
St. Vincent	*Acropora*	SA	13	24
St. Vincent	*Montastraea*	SM	14	21
Total			82	211

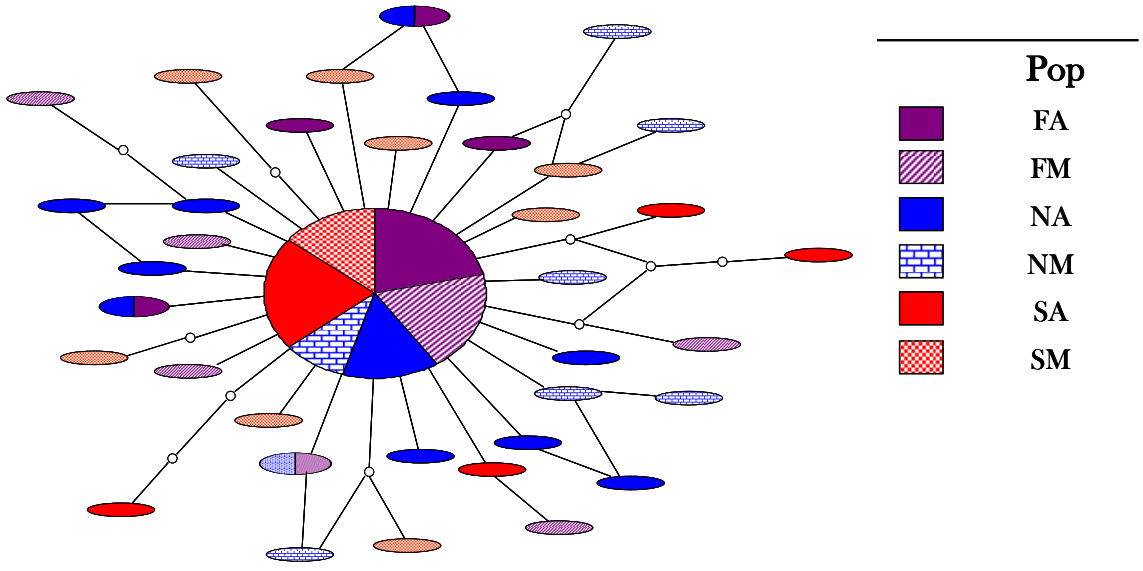

Fig. 5. 95% parsimony network for Cytochrome b gene. Each oval represents a single haplotype. The size of the oval corresponds to the frequency of that haplotype. Branches represent single point-mutations. Open circles represent missing or theoretical haplotypes. Haplotypes do not cluster by coral host taxa or geographical location.

Table 5. Pairwise F_{ST} values for microsatellite. A F_{ST} value of zero indicates no differentiation whereas a F_{ST} of 1 indicates complete differentiation. No values were significantly greater than zero.

	CA	CM	FA	FM	NA	NM	PMY	SA	SM
CA	_								
CM	0.003	_							
FA	0.0004	0.0031	_						
FM	-0.0028	0.0011	-0.0021	_					
NA	-0.005	0.0025	-0.0037	-0.0023	_				
NM	0.0009	0.0085	-0.0013	-0.0049	-0.0029	_			
PMY	0.0044	0.0016	0.0054	-0.002	0.0045	-0.0006	_		
SA	-0.0002	0.0062	-0.0027	-0.0034	-0.0071	0.0001	0.0054	_	
SM	0.0045	0.0002	0.0016	0.0009	0.0006	0.0043	0.0029	0.0037	_

Outreach and Dissemination of Information

Information on this project is available at the NOAA Southeast Fisheries Science Center website (http://www.sefsc.noaa.gov/bearprojects.jsp). Data has also been presented at the following scientific conferences:

Johnston L, Baums IB, Miller MW (2008) Plasticity of the Corallivorous Gastropod *Coralliophila abbreviata:* Implications for Imperiled Caribbean Corals. Oral presentation. 11th International Coral Reef Symposium, Ft. Lauderdale, FL

Johnston L, Baums IB, and Miller MW (2007) Plasticity of the corallivorous gastropod *Coralliophila abbreviata*: Implications for imperiled Caribbean corals. Poster presentation. 3rd International Conservation Genetics Symposium. New York, NY

Johnston L, Baums IB, and Miller MW (2007) Addressing cryptic speciation and population structure of the corallivorous gastropod *Coralliophila abbreviata.* Oral presentation. Benthic Ecology Meeting, Atlanta, GA

The following two scientific papers will be submitted to peer reviewed scientific journals in the near future:

Johnston L, Baums IB (in prep) Isolation and characterization of microsatellite loci of the corallivorous gastropod *Coralliophila abbreviata* and their cross-amplification in the congener *C. caribaea.*

Johnston L, Baums IB, and Miller MW (in prep) Caribbean wide population genetic structure and connectivity of the corallivorous *Coralliophila abbreviata*

Furthermore, one masters level graduate student at RSMAS was trained in molecular techniques during the course of this study.

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